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EXPERIMENTAL STREPTOCOCCUS PNEUMONIA AND EMPYEMA. III.

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In previous articles[1] we have described a form of experimental streptococcus empyema which can readily be produced in rabbits by direct injection into the pleural cavity of a very small amount (0.1 to 0.2 c c of a 24-hour broth culture) of a passage strain of streptococcus pyogenes (Holman) originally derived from a case of fatal human empyema. This experimental condition in rabbits offers almost complete analogy to the human condition as regards its progress. It is a process of infection by extension proceeding from the side infected and involving the pericardium and the other pleural cavity. The blood gives positive cultures with no great regularity except after death, and no lesions occur elsewhere in the body. This empyema differs from the human condition in being more acute since it invariably terminates fatally in normal animals by the fifth day on an average.

This experimental empyema in rabbits, moreover, differs from its human analogue in its artificial method of origin. Human streptococcus empyema, which has been so frequent and so fatal following measles, influenza, and broncho-pneumonia in general, apparently originates by extension of the micro-organism, which is its causative agent, downward through the respiratory tract. This hemolytic streptococcus is found in the mouth and tonsils, particularly in those who later acquire bronchopneumonia and empyema. There is further evidence that the human infection proceeds very rapidly. The clinical signs of bronchopneumonia are perhaps not even detected before the patient exhibits evidence of a pleural effusion. It seemed to us desirable, therefore, when we began these studies, to endeavor to repeat experimentally, if possible, what is presumably the entire life history of this form of infection in man, and to furnish light on its exact course. Our earlier experiments in this respect were, as we have already stated, a failure. We were unable to produce the slightest result by inoculating large amounts of streptococcus culture into the trachea. In view of our

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[1] Jour. Infect. Dis., 1920, 26, p. 265; and 1921, 28, p. 1.

present success in obtaining positive results it is evident that our failures were due to several causes which we are now able to analyze by comparison.

Experimental pneumonia with the streptococcus has been produced in dogs by Wollstein and Meltzer[2] and in a few instances by Graham and Bell. In a series of articles beginning in 1919, Wollstein and Meltzer describe the production of a form of bronchopneumonia in dogs produced by means of intrabronchial insufflation. The infection was in no instance fatal in their first two series of animals, but gave rise, as was evident by sacrificing them, to a characteristic form of bronchopneumonia which we will later describe in more detail, and which differs from the form of pneumonia produced by a similar inoculation of pneumococcus. In a more recent article,[3] these same authors obtained a mortality of 41.5% in 24 dogs by using a strain of streptococcus derived from the same series of human empyema cases as the one with which we have worked. This micro-organism, moreover, invaded the blood stream regularly and produced empyema in 12% of the cases, in this respect differing from the two previous strains of streptococcus employed.

Graham and Bell[4] have likewise obtained lobular pneumonia followed by empyema and pericarditis in 3 of 6 dogs which were given insufflation of from 2 to 5 c c of empyema fluid from a dog that had been inoculated directly into the pleural cavity.

In a recent article, Blake and Cecil[5] have produced lobular pneumonia of an interstitial type in 9 monkeys by intratracheal injection of various doses of streptococcus hemolyticus. Death occurred in 3 animals that had received the larger doses (10 c c), and was accompanied by a serofibrinous pleurisy.

PERSONAL EXPERIMENTS

We have now succeeded in producing bronchopneumonia followed by empyema in every one of the 18 rabbits in which we have attempted it, in the following manner: The rabbits are etherized, and by means of a soft rubber catheter pushed down the trachea, amounts of culture varying from 0.01 c c (usually diluted to 2 to 3 c c volume in plain broth) up to 10 c c have been employed. The catheter is pushed down into the larger bronchi as far as it will go and the culture fluid injected from a pipet and followed by forcing in a small volume of air. These cultures were not only of the passage strain used in the production of empyema by direct inoculation ,but have been grown for the purpose of this intrabronchial insufflation in 5% rabbit blood broth. Although larger amounts of 4, 6 and 10 c c apparently produced more rapid death, smaller doses of 1 c c and less have also been almost invariably fatal.

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[2] Jour. Exper. Med., 1912, 16, p. 126; ibid., 1913, 18, p. 548.

[3] Proc. Soc. Exper. Biol. and Med., 1918, 16, p. 40.

[4] Amer. Jour. of Med. Sc., 1918, 156, p. 839.

[5] Jour. Exp. Med., 1920, 32, p. 4.

In a series of 18 animals 2 were killed 24 and 48 hours after injection, and the others were allowed to proceed to a fatal termination, which occurred in all but one instance, and that with the smallest dose (0.01 c c), in from 1 to 5 days. In all instances, including those dead in 24 hours, marked exudative pleuritis was present, and in all but 3 of these cases the pleurisy was bilateral. The exudate resembles in all respects the one we have described following intrapleural inoculation; that is to say, it is at first serous, then serofibrinous, then serofibrino-purulent in character, and varies in amount from 5 to 25 c c in each pleural cavity. In all but the earliest one of our series pericarditis was also present.

The lungs in this series of animals are not as compressed as in the series obtained with intrapleural inoculations. They are not found collapsed and atelectatic when the cavity is opened, but are dark red and in patches firm in consistency. They may readily be insufflated through the trachea in certain areas leaving firm airless areas which may be simply congested or are definitely consolidated on section. The distribution of these firm areas is irregular, appears in one or both lungs, and tends in 3 to 5 days to become grayish rather than dark red in appearance.

It is apparently necessary to proceed more or less as we have outlined in order to produce positive results. We have found, for example, that simple intratracheal injections of even large amounts of from 10 to 30 c c of our same passage culture made by means of a hypodermic needle between the cartilages of the trachea, will produce no results beyond a local infection. Four negative experiments of this sort were tried. In one of two instances, we did not succeed in producing pneumonia by means of intrabronchial insufflation of 1 c c of our passage culture grown in plain broth instead of blood broth. In the one instance tried serum broth gave the same positive result as blood broth. Although it is true that there are more organisms present in serum broth or blood broth cultures of the same age, we should attribute positive results under the latter conditions to the medium employed since we have succeeded with very small amounts of the blood-broth culture. Doses of 0.2, 0.02 and one of two doses of 0.01 c c of a 24 hour blood-broth culture, diluted to 3 c c in plain broth, have given positive results.

Microscopic examination of tissues from these animals shows the following stages in the evolution of this process: In 24 to 48 hours, thrombosis of bronchial blood vessels may be found. Desquamation and destruction of both bronchial and alveolar epithelium is evident. Perivascular and peribronchial edema is present. The alveoli are filled in localized areas, often in well marked peribronchial situation, for the most part by desquamated alveolar epithelial cells, but on the 2d to 4th days polymorphonuclear leukocytes to a large extent replace these epithelial cells. Areas of necrosis appear on the 4th day, and from the 3rd to the 5th day well demarcated infarcts may occur, surrounded by zones of infiltration of red blood cells and leukocytes. The alveoli and bronchioles continue to be involved and filled with serum, fibrin and cells. From the 3d to the 4th day the perivascular and peribronchial edema become definitely infiltrated by mononuclear and polymorphonuclear cells, thus suggesting the interstitial bronchopneumonia described by MacCallum [6] in the human cases.

It should be noted that this appearance agrees with the one given by Wollstein and Meltzer [2] in their original communication, and does not differ notably from the one described by Blake and Cecil in monkeys. The essential points in this process and its specificity may well be contrasted with the parallel studies that we have made in a few animals insufflated by the same method with type 1 pneumococcus.

The work on experimental pneumococcus pneumonia is older and more extensive than that on pneumonia produced by the streptococcus. Disregarding the earlier literature in which the authenticity of cultures would naturally be under suspicion, we find that in recent years pneumonia has been produced by means of the pneumococcus in dogs, rabbits and monkeys. The work of Wadsworth,[7] which seems to have inaugurated this recent phase of investigation, is somewhat indefinite as regards the results produced. At all events, the introduction of the method of intrabronchial insufflation by Lamar and Meltzer[8] led to the first complete study of pneumococcus pneumonia in dogs, followed in the same year by a less complete article by Riesman and Kolmer [9] and another one by Wollstein and Meltzer[10] in the following year. In 1918 Wadsworth[11] reviewed his own work in this field. Whereas Lamar and Meltzer[8] had a mortality of 16%, Wadsworth obtained no fatal results, and the results of Riesman and Kolmer lie intermediate between the two.

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[6] Rockefeller Institute, Monograph No. 10, 1919.
[7] Amer. Jour. of Med. Sc., 1904, 127, p. 851.
[8] Jour. Exper. Med., 1912, 15, p. 133.
[9] Trans. Assn. Amer. Phys., 1912, 27, p. 193

Pneumococcus pneumonia has been studied in the rabbit by Winternitz and Hirschfelder[12] and by Klein and Winternitz,[13] who utilized the method of insufflation described by Lamar and Meltzer and obtained a high mortality (85%). More recently Blake and Cecil[14] have described pneumococcus pneumonia in monkeys in a series of articles dealing with pathogenesis and modes of invasion by this micro-organism.

In all these articles the pneumonia described is definitely of the lobar type, both in dogs and rabbits. Pleurisy when described has usually been localized and without effusion (Lamar and Meltzer;[8] Winternitz and Hirschfelder[12]). In most instances the micro-organism has been found in the blood early in the course of the disease.

As a check on the specificity of streptococcus pneumonia, we have inoculated a small series of rabbits by the same intrabronchial method with a culture of type 1 pneumococcus. Our results are similar to those described by Winternitz and Hirschfelder.[12] The eight animals that were inoculated all died within 3 days, apparently of a septicemia, as the blood cultures were uniformly positive when taken during life. The findings in the lung offer certain interesting contrasts to those following streptococcus inoculation. The consolidation produced was at once more extensive and uniform. One or more lobes were in all cases affected, and the distribution of the consolidation was usually throughout the entire lobe or lobes affected. Certain small areas, however, could usually be insufflated.

In one of the eight instances there was a definite pleuritis with effusion, which is in sharp contrast to the uniform and extensive exudate in the streptococcus cases. In 2 of 8 animals examined within the first 24 hours, a slight serous exudate was found, but in the other 6, which died later, there was no pleurisy with effusion, and in only one was a localized dry pleurisy of limited extent observed. A slight serous pericarditis was present in one of the 8 cases.

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[10] Jour Exper. Med., 1913, 17, p. 353.
[11] Jour. Med. Res., 1918, 39, p. 147.
[12] Jour. Exper. Med., 1913, 17, p. 657
[13] Ibid., 1915, 21, pp. 304 and 311.
[14] Ibid., 1920, 31, pp. 403, 445 and 499.

The microscopic findings in the involved areas are quite distinct from the lesions produced by the streptococcus. Animals dead during the first day show an extreme congestion of the blood vessels. The alveoli are filled with serum, fibrin, and red blood corpuscles. The alveolar and bronchial epithelium remains relatively intact. On the 2d day definite gray hepatization is evident, many alveoli being packed with polymorphonuclear leukocytes, red blood cells and a few epithelial cells. A limited bronchiolitis appears. In animals dying on the 3d day the vessels remain extremely congested, but most of the alveoli have become clear and contain at most small amounts of fibrin and serum. There is a slight peribronchial and perivascular infiltration of mononuclear cells. In other words, the process is apparently in course of resolution and shows none of the necrotic areas present in the streptococcus pneumonia. Death evidently is due to the septicemia already mentioned, which it should be recalled occurs irregularly in the streptococcus infections.

THE ROUTE OF INFECTION IN EXPERIMENTAL STREPTOCOCCUS PNEUMONIA AND EMPYEMA

We are now in a position to consider more attentively the actual course of infection in this experimental empyema, which we have produced by a natural route such as the one it presumably follows in human beings. As we have seen, the introduction of small amounts of our streptococcus culture into the bronchi leads very rapidly to involvement of the lungs in the form of a characteristic broncho or lobular pneumonia which presents many analogies to the human condition, which is further paralleled by the rapid appearance of a pleurisy with effusion. As we have noted, rabbits killed or dying from 24 hours on show a profuse pleural exudate quite different from the negative or localized sequels of pneumococcus infection.

A study of the streptococcus in the tissues by means of the Gram-Weigert stain shows that, whereas the organisms are diffusely distributed in the alveoli and alveolar walls after inoculation, they soon become collected in the perivascular lymphatics, and are found within 24 hours in the pleural exudate on the surface of the lung.

The exact route of extension of the streptococcus in human infections of this type has been studied fully by MacCallum[6] and involves rather fundamental considerations as to the finer anatomy of the lung lymphatics. Miller,[15] on the basis of his study, has concluded that the lymphatics of the lung run in two directions, mainly from the septum and the walls of the blood vessels toward the hilum where they empty into the bronchial lymph nodes. In the second place, Miller states that the current to the superficial layer of the lung is directed away from the hilum and toward the pleural network, in evidence of which he demonstrated valves that open in this direction. MacCallum,[6] on the other hand, on the basis of his observations thinks "that in the lymphatic apparatus of the lung the natural current is from the pleural surface toward the hilum of the lung." The route of pleural involvement, then, according to MacCallum, although through the lymphatics, would be against the stream, and is brought about by thrombosis of these lymphatics and propagation of bacterial growth through the thrombus. This route would certainly delay the passage of streptococcus from the alveoli into the pleural cavity appreciably.

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[15] Amer. Jour. of Roentgenology, 1917, 4, 269; Amer. Jour. of Anat., 1905, IV, 445.

The present day interest in these actual routes of infection through the respiratory tract and the lungs to the pleura is again brought out by recent work of Winternitz, Smith and Robinson,[16] which indicates that the pathway of infection from the upper respiratory tract into the lung may be by means of the subepithelial lymphatics of the larger bronchi through the nodes at the hilum of the lung and thence through the lymphatics into the lung tissue rather than directly through the bronchioles and alveoli.

These questions are particularly susceptible of experimental study by means of this type of infection in the rabbit that we have described. In the first place, it would not seem evident from a superficial study that the route of infection suggested by Winternitz, Smith and Robinson is a usual one. As we have already stated, we have found it impossible, as in the past others have done, to produce pneumonia with the streptococcus or the pneumococcus by means of simple intratracheal injection, which would certainly allow for absorption through bronchial lymphatics. We have, moreover, endeavored to produce positive results by inserting the needle under the epithelium of the trachea but with equally negative results. Apparently, the deposition of the streptococcus in the smaller bronchi and alveoli is necessary in order to obtain results. When we came to study more minutely the evolution of our experimental empyema through the lung, we had on hand an interesting series of tissues for comparison. The form of empyema in which we have extensively studied various possibilities of vaccine, serum, and dye therapy has been produced uniformly by injection of a very small amount of broth culture directly into the pleura. With our test dose the results are invariably positive, and a fatal empyema occurs. The appearance of the lungs in the empyema produced by intrapleural inoculation is, however, quite different from the condition found when the empyema is subsequent to a streptococcus pneumonia following intrabronchial insufflation. Following the intrapleural inoculations, the lungs are found extremely atelectatic and reduced to the smallest possible space as contrasted with rather full noncollapsible lungs in the conditions of pneumonia which we have just described. In the first instance, moreover, the lungs can be expanded by insufflation with air after removal from the chest cavity, even after they have been compressed by the pleural fluid for a number of days. On the other hand, the lungs of the pneumonia rabbits, or at least definite areas in them, are incapable of expansion.

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[16] Bull. Johns Hopkins Hosp., 1920, 31, p. 63.

The greatest interest lies in the localization of the bacteria in the routes of infection which we are studying. Whereas in the pneumonia the streptococci are diffusely scattered throughout the lungs in the beginning and later concentrated in the lymphatics and pleura, following the empyema produced by intrapleural inoculation, no micro-organisms are found below the layers of fibrin on the surface of the lung. In a series of animals that died from intrapleural injections at intervals of from 1 to 28 days (the latter a partially immunized rabbit) and in which the sections were studied microscopically, in no instance were any micro-organisms found within the lung tissue itself. Various degrees of compression of the alveoli and a congestion were evident, and in the later stages (10-28 days) connective tissue replaced lung tissue proper for the most part. It is evident, then, that the streptococci extend rapidly from the lungs into the pleura, but not in the opposite direction. There are, of course, mechanical reasons to explain this fact. Principally concerned is the negative intrathoracic pressure and the fact that the lung on which pleural fluid presses does not expand normally. It would seem, however, that if the lymphatics open from the pleura toward the hilum, as MacCallum asserts, that at least in the early stages following intrapleural inoculation, streptococci would be demonstrable in the lung tissue. During the early hours after inoculation the lung continues to expand normally since very little fluid accumulates, as can be shown by the fact that thoracentesis is usually negative in the first 12 hours after injection.

We have undertaken to determine the rapidity with which streptococci pass from the lungs into the pleural cavity in rabbits by means of an artificial respiration chamber. The apparatus which we have independently devised and employed is similar to that recently described by Graham.[17] The lung of a normal rabbit freshly removed from the body is suspended in a vacuum bottle and connected with a negative water pressure apparatus so devised that by opening and closing a pinch cock the pressure may be made to increase or decrease rhythmically. The trachea of the suspended lung is tightly tied about a glass tube which protrudes through a cork to the exterior air. The lung is found to expand and collapse in this chamber to all appearances in a manner similar to that in the body.

If a small amount of fluid is introduced through the cannula into the trachea, an exudate appears rapidly on the surface of the lung, being greatest, according to Graham, during expiration. In our experiments, the negative pressure which was allowed to effect the expansion of the lung did not exceed 6 mm. of mercury.

If care is taken to remove a lung with aseptic precautions and to prepare it for the chamber as described, cultures taken from the surface are found to be sterile. If then, after 1 or 2 expansions of the lung in the chamber, 2 to 5 c c of a broth culture of streptococcus are introduced into the trachea and respiration continued for 2 to 5 minutes, cultures from the surface of either or both lobes give colonies of streptococcus when streaked on blood agar. It would appear, then, from such experiments that the streptococcus may reach the pleural cavity through the lung in a matter of minutes, and a free opening of lymphatics toward the pleura would seem to be indicated.

Further consideration, however, has shown us how erroneous it would be to draw conclusions from an experiment of this sort. As we have indicated, the artificial respiration chamber would seem to reproduce natural conditions with fidelity. Our suspicions were awakened, however, in one of our experiments in which through inadvertence the lung was allowed to expand to an abnormal degree. Was it not possible that even in the carefully watched experiments in which the pressure was measured, we might have ruptured the epithelium of the pleura or in some other manner made the passage of bacteria easier than under normal conditions? It obviously became necessary to kill a series of animals at stated periods after intrabronchial insufflation in order to determine within certain wide limits how long it takes for the injected bacteria to reach the pleural cavity under natural conditions of infection. We were then surprised to find that cultures from both pleural cavities remain sterile ½ hour and 6 hours after insufflation. An animal killed 12 hours after insufflation had one lung that was firm and hemorrhagic with a bloody serous exudate measuring a little over 1 c c and containing numerous streptococci. It is evident, then, that the involvement of the pleura in our experimental streptococcus pneumonia is a matter of several hours rather than of minutes as seemed demonstrated by the in vitro experiments.

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[17] Jour. Am. Med. Assn., 1921, 76, p. 784.

CONCLUSIONS

Experimental pneumonia may be produced in the rabbit by bronchial insufflation of very small amounts of a passage culture of hemolytic streptococcus. Histologically this pneumonia is lobular in distribution, necrotizing in effect, does not resolve readily, and is characterized by peribronchial and perivascular edema and later infiltration of mononuclear cells (interstitial bronchopneumonia). It is quite different in character from the pneumonia produced by the pneumococcus.

These differences are further marked by the occurrence of pleurisy with effusion, involving by extension both pleural cavities and the pericardium, in the streptococcus infection. On the other hand, septicemia is the rule with the pneumococcus infection but not with the streptococcus. Both forms resulted fatally.

The natural route of infection with the streptococcus seems to be from the alveoli to the pleura, rather than by the lymphatics of the larger bronchi. When injections are made into the pleural cavity, the micro-organisms never penetrate through the pleura into the lung tissue. This would militate against the idea of a lymphatic stream from pleura to hilum.

Experiments with an artificial respiration chamber seemed to prove that the streptococcus passes from the lungs to the surface of the pleura in a few minutes. It is evident, however that conclusions derived from such experiments cannot explain conditions in the living body where it is found that involvement of the pleura takes place in a matter of hours (6 to 12) rather than of minutes.